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# The Effect of Maturation Status on Fatty Acid Profile of *Xanthium strumarium* L. Fruits Oil

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**ABSTRACT**

Cocklebur is a very precious medicinal plant due to its biochemical contents with antioxidant, antimicrobial, and antioxidants. It can also be evaluated as a source of energy. Cocklebur seeds and seed oil is not edible and contains 67% more biomass than cotton. Therefore, it can be evaluated as a sustainable energy source. Recently, the use of cocklebur as biodiesel makes this plant a point of interest for researchers. However, more studies are needed to evaluate factors that affect the quality of this kind of oil for the desired application. The fatty acid profile of matured and immature oil of Cocklebur whole fruits including seeds from Osmaniye province in Turkey was investigated in this study. Although the main fatty acid in the immature sample was oleic acid with 50.17%, its amount (25.96%) decreased in mature sample oil, converting linoleic acid, which is the major fatty acid of cocklebur seed oil. Mature fruit oil had a higher amount of USFA (81.34%) than immature fruit oil (56.09%). These results showed that maturation status significantly affected the concentration and rates of fatty acids in studied Cocklebur. This study will help international researchers evaluate qualified seed oils for application in industry and biodiesel production.

## Pıtrak (*Xanthium strumarium* L.) Meyve Olgunluk Seviyesinin Meyve Yağ Asitlerinin Üzerinde Etkisinin Belirlenmesi

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**ÖZ**

Pıtrak biyokimyasal içeriği ile çok değerli bir tıbbi bitki olup, enerji kaynağı olarak da değerlendirilmektedir. Son zamanlarda pıtrağın biyodizel olarak kullanılması bu bitkiyi araştırmacıların ilgi odağı haline getirmiştir. Bununla birlikte, amaca doğru uygulaması için bu tür yağların kalitesini etkileyen faktörleri değerlendirmek için daha fazla çalışmalara ihtiyaç vardır. Bu çalışmada, Türkiye'nin Osmaniye ilinden tohumları da dahil olmak üzere bütün meyvelerinin olgun ve olgunlaşmamış yağının yağ asidi profili araştırılmıştır. Olgunlaşmamış örnekte %50,17 ile oleik asit en fazla yağ asidi olmasına rağmen, olgun örnekte miktarı (%25,96) daha az bulunmuştur. Olgun meyveler olgunlaşmamış meyvelere nazaran (%56,09) daha yüksek miktarda doymamış yağ oranına (%81,34) sahiptir. Bu sonuçlar, olgunlaşma durumunun pıtrak meyve yağ asitlerinin konsantrasyonunu ve oranlarını önemli ölçüde etkilediğini göstermiştir. Bu çalışma, hem endüstride hem de biyodizel üretiminde uygulama için nitelikli tohum yağlarının değerlendirilmesi konusunda uluslararası araştırmacılara ışık tutacaktır.

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## 1. Introduction

Increasing population growth and demands for fuels encounter the humans being with significant challenges. About 86.7% of the basic energy in the world is obtained by fossil fuels (Genel et al., 2016). Therefore, the damage caused by fossil fuels is of concern. On the other hand, it is estimated that fossil fuels will run out in the near future. Besides, fossil fuels increase the quantity of $CO_2$ by approximately 40% (Hook and Tang, 2013). Therefore, discovering plant species with agroindustrial and bioenergetic potential and renewable energy sources has attracted great attention worldwide. Although some edible oils such as soybean, safflower, corn, and canola have been used as biodiesel, the application of these oils caused imbalance and deficiencies in human food supplies, especially in some countries (Chang et al., 2013). Evaluating the plants with unfavorable use for human consummation may be a good solution for overcoming deficiencies in edible oils (Hasheminejad et al., 2011). Therefore, countries with rich ecological diversity play a significant role in the assessment of suitable plant species which cover these criteria. By replacing renewable energy sources with fossil fuels, a range of environmental damages such as erosion, landslide, and flood can be prevented besides the improvement of dam basins and increased biodiversity (Nagel et al., 2005; Wei-He and Jiang, 2010; Cheng-Jiang et al., 2012; Genel et al., 2016). Cocklebur is among plant species that can meet these criteria. Cocklebur (*Xanthium strumarium* L.) is a self-fertilizing annual weed with approximate 20-90 cm height belonging to the Asteraceae family of which all parts of the plant including stem, leaves, roots, fruits, and seeds, are used in folk medicine for the treatment of a wide range of disease worldwide (Kamboj and Saluja, 2010; Romero et al., 2015). Previous studies have proven that phytochemicals isolated from cocklebur have high antibacterial, antifungal, antileishmanial, antitrypanosomal, and antioxidant activity and also have anthelmintic, antiulcerogenic, diuretic and anticancer, antitumor, antitussive, anti-inflammatory, analgesic, antimitotic, and very strong hypoglycemic effects (Kamboj and Saluja, 2010; Kumar and Rajkapoor, 2010; Patil et al., 2012; Aranjani et al., 2013). Glycosides and phytosterols compounds present in cocklebur fruits are also used to treat diseases such as allergic rhinitis, sinusitis, urticaria, catarrh, rheumatism, rheumatoid arthritis, constipation, diarrhea, lumbago, leprosy, pruritis, and smallpox. The extraction of the seeds remedies the bladder disorders and open sores (Kamboj and Saluja, 2010).

*Xanthium strumarium* is located between 53° north and 33° south latitude (Eymirli and Torun, 2015). Although it is prevalent in temperate regions, it is also found in regions where subtropical and tropical climates prevail (Holm et al., 1991). The plant originates in the North American continent but the south of Canada to the United States, into Mexico, is also accepted as its field homeland. Cocklebur is distributed all over the world, from America to Europe and Asia, including Russia, Iran, India, North Korea, and Japan to the Far East (Lee, 1996; Kim et al., 2003). The genus Xanthium has 30 species worldwide, but only three species (*X. orientale* L., *X. spinosum* L. and *X. strumarium* L.) are common in Turkey (Uskutoglu et al., 2018).

Cocklebur has a round, stout, white-hairy stem with purple spots. The leaves are alternate and triangular-ovate with a light green color, 5-20 cm long, and three-lobed with prominent veins. The leaves have long petiole and strigose hairs on both surfaces. The capitula are in axillary racemes. The female capitula are elliptic, 2–5 mm in diameter; the male capitula are saucer-shaped, 3–5 mm in diameter. The achenes are black and oblong enclosed hooked bristles. The flowering time ranges from July to October, and the fruit ripening period may last from September to October. The monoecious flowers are white or pale green. Fruits are 1-3.5 cm in length with needle-like protrusions on the surface and have two seeds (Kamboj and Saluja, 2010; Fan et al., 2019).

The seed oil of cocklebur ranges from 20.4% to 42% based on reports from previous studies which contains about 90% unsaturated fatty acids. The most abundant fatty acid in the seed oil of cocklebur is Linoleic acid, followed by oleic acid (Chang et al., 2013; Cesur et al., 2017; 2018). Linoleic acid is the essential fatty acid that the human body cannot synthesize and has great importance in the heart's health and adjusting the cholesterol balance (Arslan, 2007). The seeds contain 35% protein (Uskutoglu et al., 2018). Therefore, the seeds of cocklebur could be evaluated as a good source of edible oil. On the other hand, erucic acid (C22: 1), which is harmful in terms of edible oil, is not observed in the fatty acid distribution of the cocklebur plant. However, the cotyledon stages and seeds (inside the bur) of cocklebur contain a toxic substance, carboxyatractyloside (CAT), which is fatal for animals and humans (Scherer and Godoy, 2014). CAT is a plant growth inhibitor, and being higher in one of two seeds causes the delay in one of the seeds dormancy. However, CAT has not been reported in adult leaves and the shell of the bur makes these organs more usable for human use. This aspect of Cocklebur seed oil makes it unfavorable for human use and makes it to be a good source for biodiesel. Pawar et al., (2022) reported that biodiesel produced from *Xanthium strumarium* L. seed oil has properties similar to other biodiesel fuels and is as per ASTM standards. However, the amount of oil and the distribution of fatty acids in the oil are the main identifiers of oil quality for biodiesel. Previous studies have proved that the profile of fatty acids may enormously change during the maturation stages (Karaca and Aytac, 2007). Canvin (1963) reported that the immature seed oil of castor beans does not contain ricinoleic acid (which is the main fatty acid of maturate seed oil with more than 80%. Baydar and Erbaş (2014) also reported that during the seed maturation process in sunflower, oleic acid decreases significantly while linoleic acid increases notably. Therefore, it was aimed to investigate the fatty acid profile of mature and immature cocklebur seed oil to evaluate it as biodiesel. To our knowledge, there is no study on the profile of whole fruit oil of mature and immature cocklebur fruit.

## 2. Materials and Methods

### 2.1. Plant Material

*Xanthium strumarium* L. fruits were collected from Osmaniye: Çona Village, Bozkele Hill, 217 m, 37˚06′03″N, 36˚19′38″E, on the 25 of October 2019. Fifteen fruits per mature and immature fruits were collected from a single tree accidentally based on maturation status. Then collected fruits were washed to remove dust and dried at 65$^{o}$C for 8 h.

## 2.2. Methods

### Oil Extraction

The oils of all samples were extracted immediately after harvest. The oil of samples was extracted via an automatic soxhlet device (Gerhardt GmbH & Co. KG). Ten grams of dried seeds were used for oil extraction. Hexane (Merck KGaA, Darmstadt, Germany) was used as a solvent, and extracted oil was weighted to determine the oil percent in the samples. The oil content of seeds was expressed as g100g-1 of dry samples. Obtained fresh oil was analyzed determination of fatty acids composition.

### Determination of Fatty Acids

Fatty acid methyl esters were determined according to the method ISO 12966- 2 (2011). One hundred milligrams of cocklebur fruit oil was mixed with 5 mL heptane and 0.5 mL of 2.0 N KOH and then vortexed. To dry, anhydrous sodium sulfate was added and left to stand for one min. The solution was used directly for gas chromatography (GC, Perkin Elmer, Auto system GLX, Shelton, USA). A Supelco SPTM-2380 (30 m 0.25 mm inner diameter, 0.25 mm film thickness) column equipped with a flame ionization detector (FID) was used for FAMs separation. The carrier gas was helium with a 0.5 mL/min flow rate. The injector temperature and detector temperature were set as 280 $^{o}$C and 260 $^{o}$C, respectively. The initial oven temperature was 120 $^{o}$C for 2 min; increased at 58C/min to 220 $^{o}$C, and held for 10 min. Data was collected and quantified with a TotalChrom Navigator, and the results were expressed as percent concentration.

## 2.3. Statistical Analysis

The experiment was conducted as a completely randomized design using three replications from one tree with the independent sample. The results were expressed as average and standard deviation. Mann-Whitney U test was performed to reveal statistical differences between samples.

## 3. Results and Discussion

The fatty acid profile of immature and mature cocklebur whole fruit oil is presented in Table 1. The distribution of fatty acids in cocklebur fruit oil was statistically different in some fatty acids while was insignificant in others in mature and immature fruit oil, explaining the process of converting fatty acids to each other in biosynthesis pathways during seed maturation.

The fruit oil content was obtained as 5.11 and 3.64 g/100 gram in mature and immature fruit oil, respectively. Previous studies reported that oil content in different species of the Xanthium genus as %25 (Cesur et al., 2016), %42 (Chang et al., 2013), and %35 (Cesur et al., 2018), which were higher than reported in this study. The less quantity of oil obtained in this study may be due to using whole fruit instead of only seeds in oil extraction. However, previous studies emphasized that the oil content of seeds is tremendously affected by species and environmental conditions, especially during the seed-filling stage (Salimon et al., 2010; Onemli, 2012). Studies reported that adequate water during the growing period leads to efficient flowering and pollination, causing an increase in the number of seeds, seed weight, and oil content. In contrast, a dry environment with insufficient water may reduce the concentration of linolenic and linoleic acid but increases the concentration of oleic acid in oilseed crops (Alyari et al., 2000). Osmaniye is located in the eastern part of the Mediterranean Region and has hot and dry summers and warm and rainy winters in which its ecological conditions dramatically affect the profile of fatty acid in both mature and immature fruit oil. Therefore, water deficit stress during seed maturation in September and October may be responsible for the low oil content obtained in this study. In agreement with previous reports, the predominant fatty acid in cocklebur ripens fruit oil was detected as linoleic acid in this study (Cesur et al., 2016; 2017; 2018) while oleic acid was in the highest concentration in immature fruit oil. However, the amount of linoleic acid in cocklebur seed oil was reported higher (Approximately %70) compared to its concentration in this study (%55) (Cesur et al., 2017; 2018). The differences may be caused by different ecological conditions mentioned above, leading to a lower concentration of linolenic acid during the dry setting fruit period besides using whole fruit for oil extraction.

The oil contents of mature cocklebur fruit oil contained 81.34% unsaturated fatty acids which is in accordance with what was reported by Cesur et al. (2017) while the concentration of UFA was much less in immature fruit oil (56.09%) with a higher concentration of saturated fatty acids (43.33%). Palmitic acid was obtained at twice higher concentrations (27.38%) in immature seed oil than in mature oil (11.11%). Besides, immature fruit oil contained heneicosanoic acid (3.18%) which was detected in minute concentration (0.05%) in mature fruit oil. Heneicosanoic acid is used in the production of foams, paints, and related viscous materials. The content of PUFA was also higher in mature fruit oil derived from high linoleic acid than in immature fruit oil which had a higher amount of oleic acid. However, the composition of fatty acids in the oil is influenced by genetic, ecological, morphological, physiological, and cultural factors. In addition, fatty acids are continuously changing during the periods from seed formation to maturity and the positions of the seeds in the plant (Karaca and Aytac, 2007) (Table 1).

Mann-Whitney U test was performed to reveal if any difference has occurred between the fatty acid levels of immature and mature cocklebur seeds (Table 2). In each individual fatty acid, significant differences were observed, excluding behenic acid (C22:0), tricosanoic acid (C23:0), Σ SFA, oleic acid (C18:1n9c)ω−9, eicosenoic acid (C20:1n9c)ω−9, linoleic acid (Cl 8:2n6c) ω−6. Besides, the changes in the total oil content and individual fatty acids by maturity can be seen in Figure 1.

**Table 1.** The fatty acid composition of Cocklebure mature and immature fruits oils [%]

| Compound | Mature | Immature |
|---|---|---|
| **Total Fat** | 5.11 | 3.64 |
| **Capric acid (C10:0)** | 0.143±0.0058 | 0.857±0.0493 |
| **Caprylic acid (C8:0)** | 0.103±0.0115 | 0.787±0.0611 |
| **Undecanoic acid (C11:0)** | 0.033±0.0058 | 0.32±0.0346 |
| **Myristic Acid (C14:0)** | 0.117±0.0058 | 0.215±0.005 |
| **Pentadecanoic acid (C15:0)** | 0.063±0.0058 | 0.095±0.005 |
| **Palmitic acid (C16:0)** | 11.117±0.0231 | 27.387±0.0907 |
| **Margaric Acid (C17:0)** | 0,080±0.000 | 0,187±0.0058 |
| **Stearic acid (C18:0)** | 4.187±0.0231 | 9.367±0.0252 |
| **Arachidic acid (C20:0)** | 0.333±0.0289 | 0.61±0.020 |
| **Heneicosanoic acid (C21:0)** | 0.047±0.0058 | 3.175±0.005 |
| **Behenic acid (C22:0)** | 1.420±0.0173 | 0.106±0.0151 |
| **Tricosanoic acid (C23:0)** | 0.247±0.0115 | 0.25±0.010 |
| **Σ SFA** | 17.870±0.03 | 43.33±0.1453 |
| **Palmitoleic acid (C16.1)ω−7** | 0.137±0.0153 | 0.247±0.0231 |
| **Oleic acid (C18:1n9c)ω−9** | 25.970±0.03 | 50.173±0.0902 |
| **Elaidic acid (C18:1n9t)** | 0.027±0.0058 | 0.12±0.020 |
| **Eicosenoic acid (C20:1n9c)ω−9** | 0.210±0.010 | 0.26±0.0361 |
| **Σ MUFA** | 26.347±0.0462 | 50.773±0.1124 |
| **Linoleic acid (Cl 8:2n6c) ω−6** | 55.373±0.0306 | 5.417±0.0611 |
| **γ-Linolenic Acid (C18:3n6) ω−6** | 0.023±0.0058 | 0.41±0.020 |
| **a-Linolenik acid (C18:3n3) ω−3** | 0.447±0.0115 | 0.113±0.0115 |
| **Σ PUFA** | 55.83±0.0173 | 5.927±0.0416 |

SFA: Saturated Fatty Acid, MUFA: Mono Unsaturated Fatty Acid,
PUFA: Poly Unsaturated Fatty Acid

**Table 2.** Mann-Whitney U test results

| Fatty Acids | Mann-Whitney U | Wilcoxon W | Z | Asymp. Sig. (2-tailed) |
|---|---|---|---|---|
| Capric acid (C10:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Caprylic acid (C8:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Undecanoic acid (C11:0) | 0.000 | 6.000 | -2.023 | 0.043 |
| Myristic Acid (C14:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Pentadecanoic acid (C15:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Palmitic acid (C16:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Margaric Acid (C17:0) | 0.000 | 6.000 | -2.121 | 0.034 |

| | | | | |
|---|---|---|---|---|
| Stearic acid (C18:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Arachidic acid (C20:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Heneicosanoic acid (C21:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Behenic acid (C22:0) | 0.000 | 6.000 | -1.993 | 0.046 |
| Tricosanoic acid (C23:0) | 3.500 | 9.500 | -0.471 | 0.637 |
| **Σ SFA** | **0.000** | **6.000** | **-1.964** | **0.050** |
| Palmitoleic acid (C16.1)ω−7 | 0.000 | 6.000 | -1.993 | 0.046 |
| Oleic acid (C18:1n9c)ω−9 | 0.000 | 6.000 | -1.964 | 0.050 |
| Elaidic acid (C18:1n9t) | 0.000 | 6.000 | -1.993 | 0.046 |
| Eicosenoic acid (C20:1n9c)ω−9 | 0.000 | 6.000 | -1.964 | 0.050 |
| **Σ MUFA** | **0.000** | **6.000** | **-1.993** | **0.046** |
| Linoleic acid (Cl 8:2n6c) ω−6 | 0.000 | 6.000 | -1.964 | 0.050 |
| γ-Linolenic Acid (C18:3n6) ω−6 | 0.000 | 6.000 | -1.993 | 0.046 |
| a-Linolenik acid (C18:3n3) ω−3 | 0.000 | 6.000 | -2.023 | 0.043 |
| **Σ PUFA** | **0.000** | **6.000** | **-1.993** | **0.046** |

Grouping Variable: immature and mature seeds

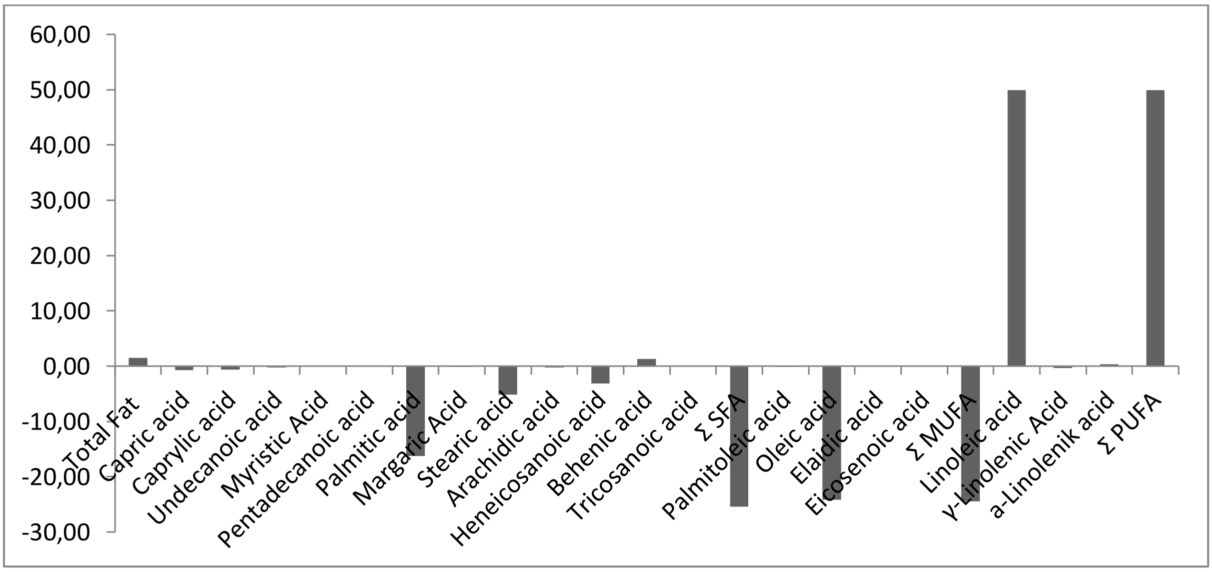


**Figure 1**. % changes of the total oil content and individual fatty acids (mature-immature).

## 4. Conclusion

Cocklebur is a precious plant in terms of medicinal application and suitable oil quality for considering as biodiesel and grows almost everywhere in the world because it has high competitive power and endurance to various climate and soil conditions. The oil content had a high concentration of linoleic acid. Mature and immature fruit oil exhibited considerable differences in fatty acid profile. Although studies have been done on seed oil of cocklebur, there is neither a study on the fatty acid profile of whole fruit nor a comparison of the fatty acids in different maturation stages. Therefore, this study will help international researchers evaluate qualified oils from Cocklebur whole fruit for application in industry and biodiesel production.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Authors' contributions

MZ and ZE contributed to the project idea, design and trials and laboratory analysis. MZ wrote the study. The article was reviewed by all authors.

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